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Durum Wheat Grain Quality Traits as Affected by Nitrogen Fertilization Sources under Mediterranean Rainfed Conditions

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Abstract. Nitrogen application, environmental variation and particularly water deficit and terminal heat that prevail during post grain filling period could significantly affect not only grain yield ability but also quality related traits of durum wheat. A field trial was conducted to evaluate the effect of two nitrogen sources applied at different rates ranging from 0 to 93.8 KgN^{-1} on yield and grain quality of three durum wheat cultivars. Increased N level from both nitrogen sources (ammonium sulfate nitrate: ASN and urea N) appeared to positively improve yield and grain quality. This effect was particularly significant when for N level superior to 40.2KgNha^{-1}. The average yield increase under maximum N level ranged from 3.23 to 3.37tha^{-1}for urea and ASN respectively. The cultivar Om Rabia appeared to better valorize nitrogen supply and was found associated with higher yielding ability of 1.78tha^{-1}, greater test weight 78.90kg/hl, grain protein content 12.43%, and gluten content 15.20%. This cultivar showed reduced yellow berry of 7.43% under N optimum application. Greater improvements were obtained for ASN than urea for all measured traits. The percentage increases were 6.09% for GY, 2.92% for TW, 4.48% for GP, 5.64% for Gl and 6.88% for CP. These results support that nitrogen derived from ASN and when its application rate is superior to 40.2 KgNha^{-1} would promote grain yield and quality of durum wheat under rainfall conditions.

Key Words: Ammonium sulfate nitrate, urea, efficient nitrogen source, grain quality, yield

Abbreviations: ASN, Ammonium sulfate nitrate;GY, Grain yield;TW, test weight;YB, yellow berry;GP, grain proteincontent

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Background

Durum wheat (*Triticum turgidum* var. durum) is the main cultivated cereal crop in Mediterranean area, covering up to 2/3 of total world production (Nachit 1998; Habash et al. 2009). Pasta, bread and couscous are among the most common derived end products from durum wheat. These products constitute the main staple food in North Africa and for many world areas. However, durum wheat productivity is limited and ranged from 0 to 6Tha^{-1} (Nachit and Elouafi 2004). Drought, variable temperatures particularly terminal heat, and low soil fertility are considered the most limiting factors that affect grain yield and grain quality (Nachit 1998; Verma et al. 1998; Lòpez-Bellido et al. 2001). However, genotype x environment interaction effects and common cultural practices would affect both water and nitrogen use efficiencies (Gate 1995; Chandrasekaran et al. 2010).

Grain protein content is considered as the main characteristic of durum wheat grain quality (Ottman et al.2000; Clarke 2001). Protein content was associated mainly to soil N fertility status (Ottman et al. 2000), resulting in an increase of gliadins and glutenins (Dupont and Altenbach, 2003). Available soil nitrogen is often insufficient and mineral fertilizers should be supplemented. In fact, Metwally and Khamis (1998) indicated that, wheat N requirements could not be met by the separate application of any organic source. In practice, Nitrogen is the most cost efficient and practical factor to manage (Wuest and Cassman, 1992).

Nitrogen application improves grain protein content of soft wheat (Warraich et al. 2002) and durum wheat (Ottman et al. 2000). Limited investigations indicated that positive correlation between nitrogen applications and the rate of grain filling were reported (Warraich et al. 2002; Hussain et al. 2006). Improving grain protein would imply the improvement of translocation and nitrogen transfer and nitrogen source-sink relationship. This is because nitrogen reprensents 7% of total dry matter in plants and it is one of the principal cell components of nucleic acids, aminoacids, enzymes, and photosynthetic pigments (Bungard et al. 1999; Brown 2000; Selim 2004).Therefore, it is assumed that nitrogen would affect most of the quality parameters such as: test weight; grain protein content; yellow berry; gluten and carotenoids pigment under rainfed Mediterranean conditions.

This study aims to determine the effect of N sources and rates on the expression of quality related traits of various durum wheat cultivars.

Material and methods

Three local durum wheat (*Triticum turgidum* ssp *durum*) cultivars were tested: Karim, Om Rabia and Nasr (Deghaïes et al. 1999; Gharbi et al. 2000).Field experiments were conducted during the growing season 2010/11on alluvial typical soil (Table1) at Boulifa experimental station (El Kef) located in Northern Tunisia (36° 07’ N; 8° 43’ E;520m above sea level) with a mean annual rainfall of 557.3mm and average relative humidity of 54.34%, 100 kgha^{-1} of phosphorus (45%) was applied prior to sowing (Table 1). The crop received 411 mm during the overall growing season. No water stress occurred during the grain filling period with a total of 212.09mm. No heat stress was noticed with an average temperature below 25°C (Table 1). The durum wheat cultivars were sown in November 2010. Weed control was carried out manually at two periods: the first was achieved at 5 leaves growth stage and the last was realized in March.

Two nitrogen sources: Urea ($CO(NH_2)_2$; 46%N) and ASN ($NH_4NO_3(NH_4)_2SO_4$; 26%N) were tested using seven nitrogen levels: 0, 13.4, 26.8, 40.2, 53.6, 67, 80.4 and 93.8KgNha^{-1}.Rates of nitrogen fertilizers were applied at three key growth stages including30% at early tillering (Z13),40% at elongation (Z16) and 30% at 2nd node (Z32) (Zadoks et al. 1974).The experiment was designed as a split-split plot arrangement with three blocks. The subplot size was 10m^2 (10m×10m). Sowing was realized at seed density of 350 seed/m^2. To assess the effect of nitrogen sources on the three durum wheat cultivars, yield and grain quality traits were assessed. These characters included grain yield (GY), test weight (TW), yellow berry (YB), grain protein (GP), gluten (Gl) and carotenoid pigment (CP) contents.

Grain yield (GY) was harvested and collected at 14% moisture in all plots during 25th of June 2011.Test weight (TW) was obtained with a standard chondrometer. Yellow berry (YB) was determined by visual inspection of 200 kernels harvested from the two middle rows of each replication. Kernels were placed on a light table and separated into YB (opaque) and normal types. Protein content of wheat grain was determined in three replications of 1g freeze-dried grain samples by Kjeldahl method applying a factor of N x 5.70. Gluten index (Gl)was determined using 12 g of flour sample. Carotenoid pigments (CP) were determined according to a standardized method of American Association of Cereal Chemists (AACC, 1995). Extraction of β-carotene was made in water saturated butanol. Grain β-carotene content was determined by optical density at 440 nm and reported to Standard solution of β-carotene (Sigma).

Analysis of variance of grain yield and quality related traits were carried out using PROC ANOVA procedure of SAS Program 9.1(SAS Institut Inc., 1999) with the option LSD to compare means of main effects and interactions. Treatment effects were declared significant at $p < 0.05$ and LSD were reported at $p = 0.05$.

Results

Grain yield, test weight, yellow berry, grain protein, gluten and carotenoid pigment contents analysis of variance are presented in Table 3.All parameters were significantly influenced by the interactions of N level × N source and N level × cultivars ($P< 0.01$). The analysis of variance revealed significant effect of the interaction N source × cultivars × N level for test weight ($P< 0.01$) and carotenoid pigment content ($P< 0.05$).

Maximum average yield was obtained at 93.8KgNha^{-1} for both N sources. ASN was more efficient with an increase of 6.09% compared to urea. The maximum yield was obtained for the cultivar Om Rabia under maximum N rate(93.8KgNha^{-1}) with 3.93tha^{-1} and 4.08tha^{-1} for ASN and urea N sources respectively (Figure1). The genotypic variation was observed from 53.6 KgNha^{-1} to 93.8 KgNha^{-1}. Om Rabia showed the maximum average yield over all N sources and N levels (1.78tha^{-1}) followed by Karim (1.65tha^{-1}) then Nasr (1.58tha^{-1}).

Test weight of experienced durum wheat cultivars was under the effect of the interactions: N level × N source; N source × cultivars and N level × cultivars. The TW increased with N application for both N sources. In fact, the maximum TW was observed under 93.8 KgNha^{-1}for Om Rabia with an increase of 9.43% for urea and 11.49% for ASN nitrogen source compared to the control. Moreover, Om Rabia showed the maximum TW under all the N levels for urea and ASN with an average of 78.90kg/hl and it was followed consecutively by Karim (77.48kg/hl) and Nasr (77.10kg/hl). However, under ASN treatments no significant difference was noted between Karim and Nasr (Figure 2). The maximum TW was observed for the ASN nitrogen source (78.95 kg/hl) compared to urea (76.71 kg/hl). In addition, ASN effects on TW were noted mainly at 53.6 $KgNha^{-1}$ leading to an average increase of 6.55% compared to the control. However, only a 3.87% increase was observed for the maximum N fertilization level compared to 53.6 $KgNha^{-1}$.

Yellow berry percentage was controlled by both N level and source as N source × cultivars and N level × cultivars (Table 3). N fertilization reduces YB percentage under the two nitrogen sources. The maximum YB reduction was obtained under 93.8$kgNha^{-1}$ranging from 87.2% to 91.61% respectively for urea and ASN compared to the control. Moreover, 80.4$kgNha^{-1}$of ASN was sufficient to reach YB reduction of 87.94%compared to the control (Figure 3).The results of this investigation indicated that ASN nitrogen source appeared to affect positively grain quality with a reduced YB (7.39%) compared to urea (8.89%). Om Rabia showed in average for both N sources the lowest YB (7.43%) followed by Karim (8.70%) and Nasr (8.29%).

Grain protein content (GP) was under the effect of: N level × N source; N source × cultivars and N level × cultivars. GP increased significantly for the three durum wheat cultivars under N fertilization of about 50% for urea and ASN at maximum N rate compared to the control.

ASN induce the highest grain protein content (12.35%) compared to urea (11.82%). In addition, Om Rabia accumulated the highest level of proteins with an average of 12.43% followed consecutively by Karim (11.98%) and Nasr (11.82%)(Figure 4).

Gluten percentage of the three durum wheat cultivars was governed by the interactions of N level × N source; N source × cultivars and N level × cultivars (Table 3). N application from either sources enhanced gluten content in all durum wheat cultivars. The improvement of this trait ranged from 49.25 to 49.5%

for nitrogen rate of 93.8kgNha^{-1} derived from either urea or ASN respectively. At lower rate of nitrogen (40.2kgNha^{-1}), gluten content was not significantly affected by nitrogen (Figure 5). The cultivar Om Rabia was associated with greater gluten content (15.2%) over all treatments and nitrogen sources followed by Karim (14.65%) and Nasr (14.45%). Moreover, nitrogen from ASN source appeared to promote gluten content with an increase of 0.81% compared to urea. Carotenoids pigment content was significantly affected by the interaction N source × cultivars × N level (Table 3). An increase of CP ranging from 61.6 to 70% was noted for the maximum N rate derived from ASN and urea (Figure 6). Greater average CP was noted for Nasr (7.22%), while lower percentage was noted for the cultivar Karim (6.25%). Moreover, ASN nitrogen source more effective on CP (6.83%) compared to urea (6.39%).

Discussion

Derived durum wheat end products are largely affected by protein quantity and quality which are controlled by environmental factors such as rainfall, temperature and soil nitrogen availability (Lòpez-Bellido et al. 2001; Zhao et al. 2009). The prevailing growing conditions as water and temperature did not affect grain filling and yielding ability of most cultivars. The same results were noticed for bread wheat under rainfed conditions with 212.09mm precipitation during April-May period, reported by Lòpez-Bellido et al. (2001) as the most important period for grain filling. The genetic component seems to be more determinant on wheat yield and those unless effect of environment (Abad et al. 2004).

The maximum yield was observed for Om Rabia with 4.08tha^{-1} and 3.93tha^{-1} under ASN and urea respectively at a concentration of 93.8kgNha^{-1}. Under the same nitrogen rate (100kgha^{-1}), maximum durum wheat yield was obtained in irrigated conditions and under urea N source (Abad et al. 2004). Moreover, the increase of N fertilization to 120–130kgNha^{-1} in irrigated conditions increased the average durum wheat yield till 5.0tha^{-1} (Hogg and Ackerman, 1998). In the other hand, maximum bread wheat yield were observed at 150kgNha^{-1} generated from urea (Hussain et al. 2006).

It is well established, the existence of an inverse relationship between yield and grain protein content (Ottman et al. 2000; Abad et al. 2004). However, the tested wheat cultivars showed an increase in yield and grain quality under N supply. In fact, N fertilization improved grain quality through the increase of test weight, grain protein content, gluten, carotenoid pigment content and the reduction of yellow berry.

Test weight is a very important grain quality parameter for durum wheat semolina. Our results showed that N fertilization improved TW from both N sources. In addition, a concentration above 40.2Nha^{-1} of ASN has to be applied to reach the maximum test weight. TW of durum wheat was between 72.7 and 81.0kg/hl (Clarke et al. 2009) and increased with rainfall during the filling period and fell slightly as N rates increased (Lòpez-Bellido et al. 2001) which were observed mainly with ASN nitrogen source.

Yellow berry resulted from an endosperm dysfunction that gives kernels a yellowish color caused by low protein content (Sharma et al. 1983) affecting the quality of the flour and the end product such as pasta. The results showed a continuous decrease of YB under N fertilization to reach an average minimum under 93.8kgNha^{-1} of 1.21% and 2.20%, respectively for ASN and urea. Ammonium nitrate conferred the same effect on triticale YB with a considerable reduction of YB upon N fertilization of 300kgha^{-1} (Sharma et al. 1983). The results were in conformity with virtuousness standard (80%) for most durum wheat commercial varieties (Mahaut 1997). The reduced YB incidence under N fertilization was attributed to the endosperm and aleurone cells fractured around the starch granules and cytoplasm (Sharma et al. 1983). Moreover, the inverse correlation between yellow berry and grain quality parameters tested in this study was noted for grain protein content (Gate 1995). In fact, the total protein content of YB kernels was generally 1 to 1.5% lower than in normal kernels (Sharma et al. 1983).

The flour protein content is correlated to N supply in the soil (Banziger et al. 1992) and growth stage N fractioning (Saint Pierre et al. 2008). Early application of N leads to less durum wheat grain protein content than late N fertilization (Ottman et al. 2000). Grain protein content of tested durum wheat cultivars showed 50% increase with N fertilization to reach 15.53% and 15.13%, respectively for ASN and urea. The same results were obtained in durum wheat by Abad et al. (2004) with an average PC of 14.4 and 15.6% under 100-150kgNha^{-1}, which was appropriate for semolina production according to industry standards (Clarke 2001). However, the maximum grain protein content for bread wheat (14.5%) was obtained at 200kgNha^{-1} and the minimum grain protein content (8.5%) was recorded in the control (Hussain et al. 2006). Grain protein is driven from the interaction of nitrogen, water availability and temperature (Zhao et al. 2009; Giambalvo et al. 2010). In fact, climate conditions were not limiting factors for grain filling (Table 1). Lòpez-Bellido et al. (2001) showed that GP increased with rainfall in filling period up to a maximum of 80mm and an average temperature of 26-27°C.

The gluten proteins constitute about 80% of the proteins contained in mature wheat grains (Shewry et al. 1995) conferring properties of elasticity and extensibility of wheat flours (Kuktaitė 2004). Gl in tested durum wheat cultivars increased under N fertilization in non limiting weather conditions. In fact, Motzo et al. (2007) indicate that temperatures above 30°C at the end of the grain filling period have a negative effect on the gluten polymerization process causing a fall of gluten index.

Yellow pigment of flour is an important quality trait that is comprised primarily of carotenoids (Ramachandran et al. 2010).Endosperm yellow color is appreciated by consumers for semolina and pasta industry as well as for the potential health benefits associated with carotenoids, such as antioxidant activity and prevention of macular degeneration (Abdel-Aal et al. 2007). Our results showed that N fertilization improved carotenoids flour pigment content. Abad et al. (2004) found that average of carotenoids pigment content in durum wheat is ranged from 4.8 to 6.5 ppm when N increased from 0 to 200kgNha-^{1}(Abad et al. 2004).

The maximum grain quality was obtained under maximum N rate (93.8 kgNha^{-1}). Nevertheless, grain quality parameters need 200 kgNha^{-1} to reach its maximum potential for durum wheat (Abad et al. 2004) and bread wheat (Hussain et al. 2006) which constitutes an increasing risk of N leaching.

Moreover, ASN source was more efficient on grain yield and quality than urea. In fact, urea uptake is very slow and low to be monitored on wheat (Criddle et al. 1988). Moreover, urea compared to ASN was found to reduce shoot growth and induce root growth in hydroponically wheat growing plants (Mérigout et al. 2008). This difference may be attributed to the organic form of urea which needs to be hydrolyzed to allow nitrate (NO_3^-) and ammonium (NH_4^+) forms available to wheat roots (Mérigout 2006).
Nitrogen fertilization constitutes a key agronomical practice to improve yield and grain wheat quality. N fertilization effects on durum wheat should be applied at a level superior to 40.2kgNha^{-1}. In addition, the maximum nitrogen level applied in this assay seems to be inefficient to attend the maximum yield and grain quality of tested cultivars. In fact, Abad et al (2004) showed that a dose of 200kgNha^{-1} leads to maximize grain quality.

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Table 1. Precipitation (mm), relative humidity (%) and air temperature (°C) for the 2010/11 growing season at Boulifa, Tunisia.

	Precipitation (mm)	Relative humidity (%)	Temperature (°C)
September	42.41	47.4	24.8
October	40.38	58.3	19.4
November	14.72	60.6	13.0
December	27.96	69.2	8.5
January	67.57	74.4	9.2
February	42.93	66.6	8.8
Mars	44.71	62.9	12.1
April	157.47	66.8	14.4
May	54.62	48.9	22.1
June	1.02	29.9	28.1

Table 2. Soil properties at the beginning of the experiment in 2010/11 growing season at Boulifa, Tunisia.

	Soil characteristics
Sand (%)	56
Silt (%)	18
Clay (%)	26
pH	7.7
MO (%)	2.21
$CaCO_3$	16
P Olsen (mg/kg)	12
K (cmol/kg)	16
CEC (cmol/kg)	0.74
K/CEC (%)	4.1

Table 3. Analysis of variance (mean square and F test) for grain yield (GY), test weight (TW), yellow berry (Yb), grain protein (GP), gluten (Gl) and carotenoid pigment (CP) contents of three durum wheat cultivars, two N sources and eight levels.

Source of variation	df	GY	TW	YB	GP	Gl	CP
Bloc	2	13.72**	0.004ns	1.39ns	0.15ns	0.13*	0.006ns
N source	1	35.17**	179.93**	81.36**	9.20**	23.71**	7.13**
N source × Bloc	2	0.02ns	0.22*	1.16ns	0.08ns	0.07ns	0.0006ns
Cultivars	2	51.81**	43.14**	20.12**	9.57**	7.10**	13.31**
N source × Cultivars	2	1.48ns	2.98**	7.56**	0.54**	0.42**	0.03ns
N source × Cultivars × Bloc	8	0.59ns	0.05ns	1.00ns	0.32*	0.05*	0.01ns
N level	7	2330.13**	149.90**	539.11**	461.66**	98.68**	22.85**
N level × N source	7	2.79**	1.11**	3.16**	5.82**	1.29**	0.10**
N level × Cultivars	14	8.28**	0.21**	1.47**	2.28**	0.24**	0.26**
N source × Cultivars × N level	14	0.64ns	0.71**	0.80ns	0.34ns	0.03ns	0.02*
Error	84	0.51	0.06	0.62	0.01	0.02	0.01
C.V (%)		4.29	0.32	9.67	1.14	1.16	1.87
R^2		0.99	0.99	0.98	0.99	0.99	0.99

ns: not significant (P>0.05); *: F test significant (P< 0.05); **: F test significant (P< 0.01).

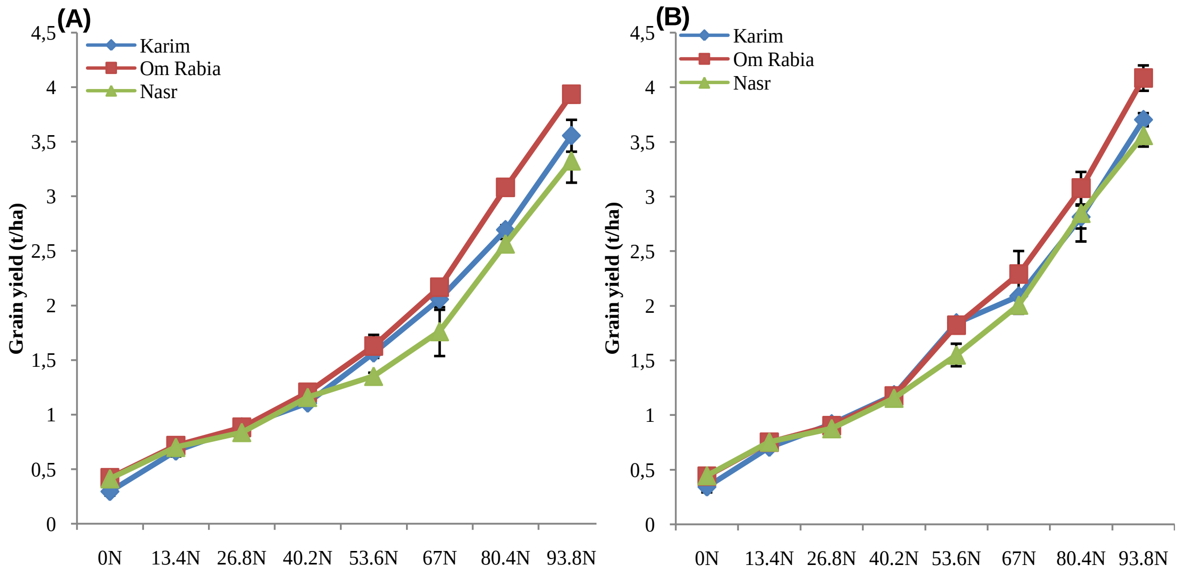

Figure 1. Variation of the grain yield (GY) of three durum wheat cultivars at eight N levels and two N sources.(A) Urea, (B) ASN.

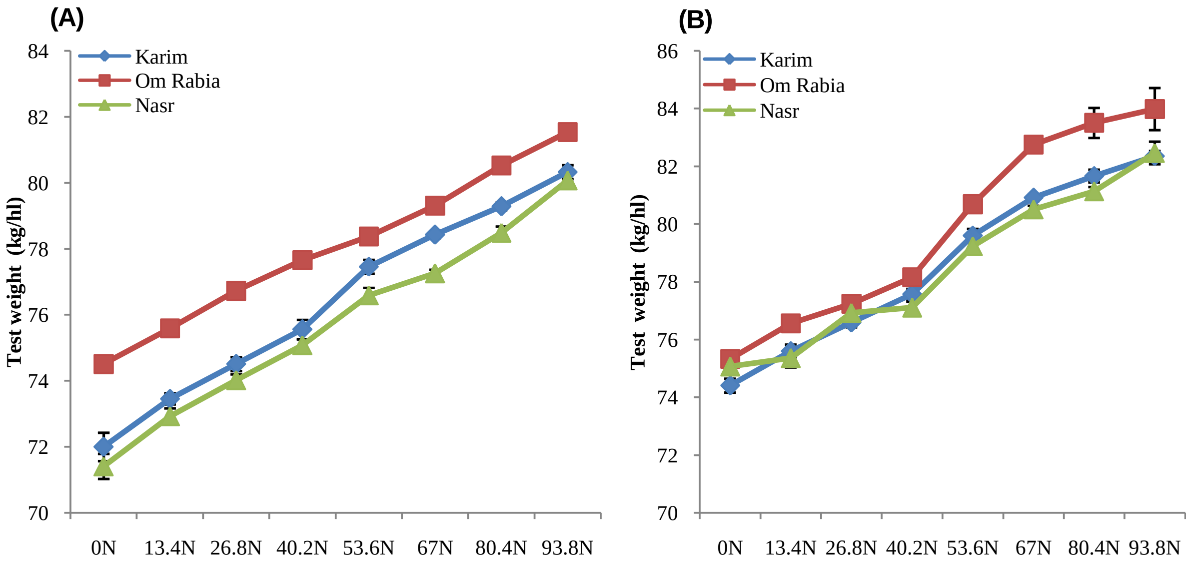

Figure 2. Variation of test weight (TW) of three durum wheat cultivars at eight N levels and two N sources. (A): Urea, (B): ASN.

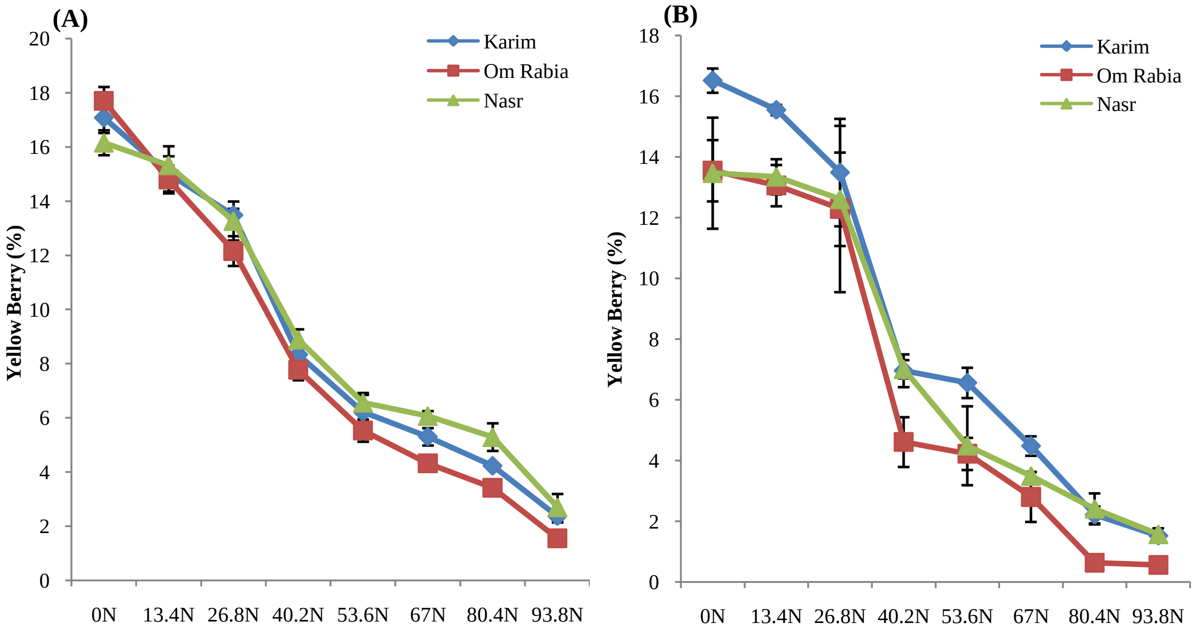

Figure 3. Variation of yellow berry (YB) of three durum wheat cultivars at eight N levels and two N sources. (A): Urea, (B): ASN.

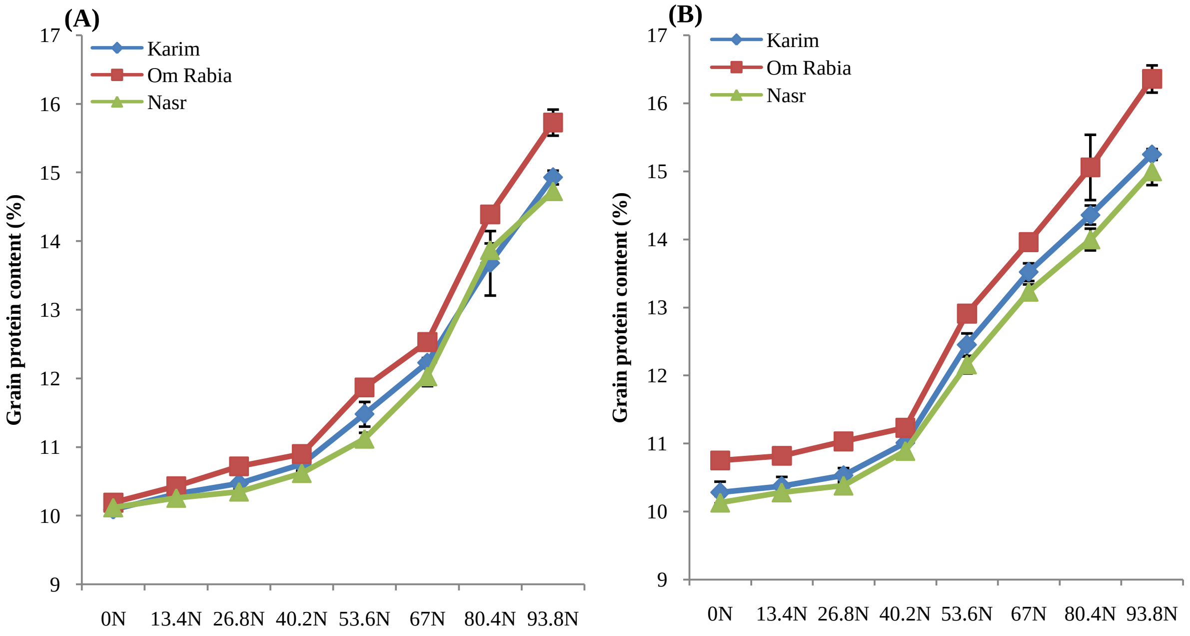

Figure 4. Variation of grain protein content (GP) of three durum wheat cultivars at eight N levels and two N sources. (A): Urea, (B): ASN.

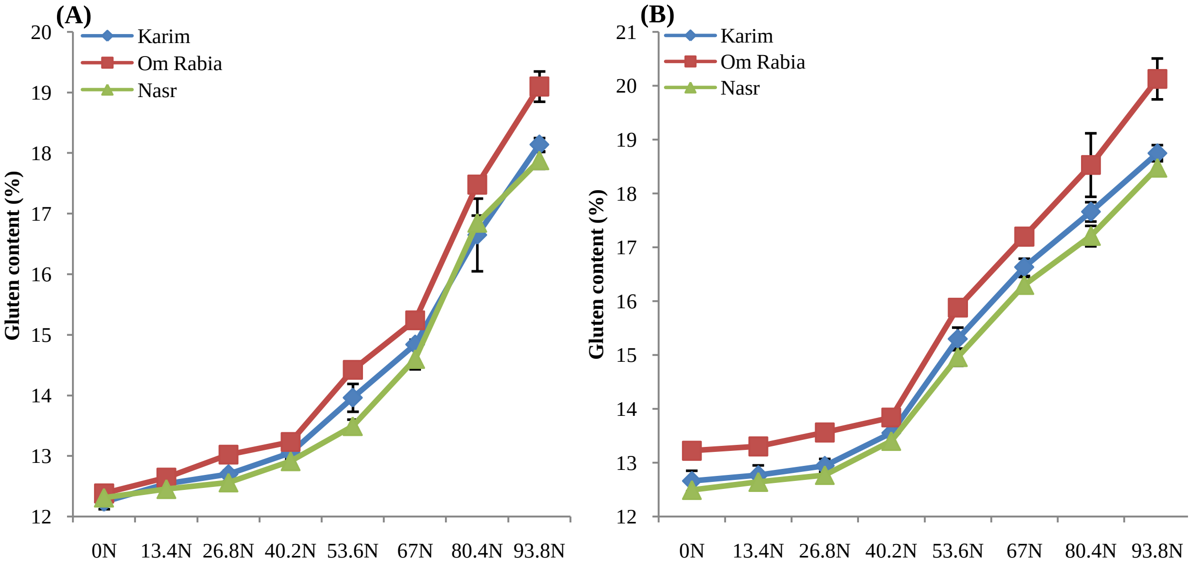

Figure 5. Variation of gluten content (Gl) of three durum wheat cultivars at eight N levels and two N sources. (A): Urea, (B): ASN.

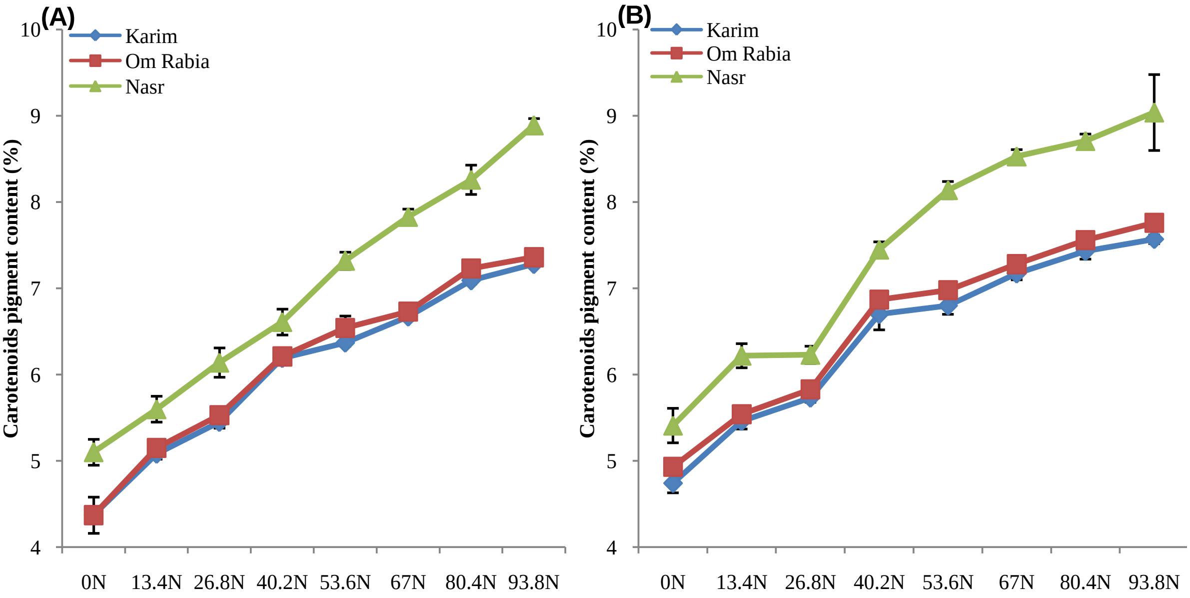

Figure 6. Variation of carotenoids pigment content (CP) of three durum wheat cultivars at eight N levels and two N sources. (A): Urea, (B): ASN.